Chromosome movement: **Kinetochores motor along**

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The equal division of chromosomes among daughter cells at mitosis involves a complex series of kinetochore-dependent chromosome movements. The kinetochore-associated CENP-E motor protein is critical for the sustained movement of chromosomes towards the metaphase plate during chromosome congression.

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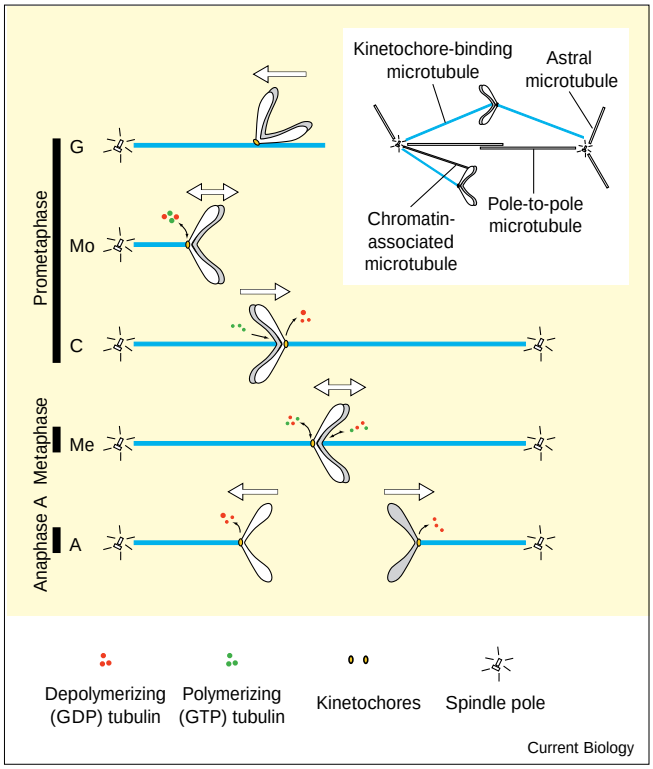
In eukaryotes, chromosome segregation occurs on a mitotic spindle formed from two polar arrays of microtubules. Chromosomes attach to the spindle microtubules by kinetochores, DNA–protein complexes that assemble on centromeric DNA. The kinetochore–microtubule linkage is very dynamic, and chromosomes move continuously during mitosis. Chromosome movement is powered by microtubule-based molecular motors, which use the energy of ATP hydrolysis to generate force, and by microtubule depolymerization, which liberates energy stored in the microtubule lattice by GTP hydrolysis during assembly [1]. A major challenge in the study of mitosis is to determine which kinetochore proteins are responsible for which aspects of chromosome movement, and to determine the relative importance of motors and microtubule dynamics in generating force. Several recent papers on the kinesin-related motor CENP-E advance our understanding of kinetochore-associated motors and illustrate the daunting technical challenges that must be overcome for chromosome movement to be understood in full.

To make sense of chromosome segregation, it is important to recall that spindle microtubules are oriented with their minus ends anchored at the spindle poles — the centrosomes — and their plus ends radiating outward. Spindle microtubules can be divided into four classes on the basis of the structures they contact (Figure 1, inset). The first class, astral microtubules, contact the cell cortex, where they play a role in orienting the spindle. The second class, pole-to-pole microtubules, overlap in the middle of the spindle with microtubules emanating from the opposite pole; motor proteins found in the overlap region cause pole-to-pole microtubules to slide relative to each other, thereby controlling the length of the spindle. The third class of microtubules interact with chromatin via chromokinesins, motor proteins on the chromosome arms which generate a polar ejection force that pushes chromosomes toward the middle of the spindle. Lastly, the fourth class of microtubules bind to kinetochores to link chromosomes to the spindle. Although all four classes of microtubules play a role in positioning chromosomes, the force for chromosome movement is generated primarily by the kinetochore–microtubule attachment.

The purpose of mitosis is to separate newly replicated chromosomes into two equal and physically distinct sets. To ensure the accuracy of this process, all pairs of sister chromatids must achieve a state of bivalent attachment prior to their poleward movement at anaphase. Bivalent attachment is achieved when one of a pair of sister chromatids is

Figure 1



Kinetochore motility and microtubule assembly/disassembly in vertebrate mitotic cells. The kinetochore of a chromosome may initially become attached to the side of a microtubule and slide along its wall (G). Once the dynamic tips of the microtubules depolymerize and attach to a kinetochore, the monovalently-attached chromosome oscillates between slow movement towards, and slow movement away from, the pole (Mo). Once the sister kinetochore becomes attached to microtubules from the opposite pole, chromosomes congress at the spindle equator (C). When the chromosome is near the equator, at the location of the metaphase plate, each sister kinetochore alternates between movement towards and away from the pole (Me) until separation at anaphase (A). (Adapted from [1].)

attached via its kinetochore to microtubules emanating from one spindle pole, and the other sister is attached to microtubules from the opposite pole. Chromosomes make such bivalent attachments by a complex series of movements, as illustrated in Figure 1 [2].

Early in prometaphase, each pair of sister chromatids attaches by its kinetochore to the wall of a single microtubule, leading to rapid (20–50 μm per minute) poleward movement (Figure 1, state G). As the kinetochore-bound microtubule depolymerizes, wall-binding matures into an end-on attachment in which the extreme (plus) end of the microtubule is found at the kinetochore. A period of slow (2 μm per minute) movement, alternating away-from and towards the pole, then ensues (Figure 1, state Mo) until collision with the end of a microtubule emanating from the opposite pole produces bivalent attachment and slow (2 μm per minute), but sustained, movement towards the spindle equator (chromosome congression; Figure 1, state C). Pairs of sister chromatids then oscillate about the spindle equator (Figure 1, state Me) until the sudden loss of sister cohesion at anaphase allows movement of individual chromatids towards opposite poles (Figure 1, state A).

A remarkable feature of all but the earliest prometaphase chromosome–microtubule attachments is that the microtubule plus ends remain associated with kinetochores. This can occur because chromosome movement is tightly coupled to microtubule dynamics so that, as a chromosome moves, microtubules attached to the leading sister chromatid shrink and microtubules associated with the trailing sister grow. The mechanisms that couple movement to microtubule assembly are not known, but kinetochore-associated motor proteins are thought to play a key role in this coupling.

CENP-E structure and localization

With the aim of determining which proteins mediate chromosome movement, there has been a sustained attempt to identify kinetochore-associated microtubule-based motors. Thus far, cytoplasmic dynein [3,4] and two kinesin-like motor proteins — MCAK/XKCM1 [5,6] and CENP-E [7] — have been localized to mammalian kinetochores. We shall focus here on CENP-E, a 312 kDa protein with an amino-terminal kinesin-like motor domain, a carboxy-terminal microtubule-binding domain and a long intervening region that is likely to form a dimeric coiled-coil [8] (Figure 2, inset). One can imagine that a motor protein with such an architecture might link apposed microtubules. The microtubule-binding activity of CENP-E is turned on only at anaphase [8], however, implying that CENP-E can crosslink microtubules only late in mitosis.

Immuno-electron microscopy and light microscopy have been used to determine the subcellular distribution of CENP-E during the cell cycle [9,10]. Early in prometaphase, just after nuclear envelope breakdown, most CENP-E is associated with microtubules. Subsequently, CENP-E becomes concentrated at kinetochores, presumably by traveling along microtubules by plus-end motor activity ([10] and see below). Kinetochore localization of CENP-E persists during metaphase and early anaphase. Late in anaphase, however, CENP-E dissociates from kinetochores, which are now near the spindle poles, and accumulates at the spindle midzone. The midzone contains the overlapping plus ends of pole-to-pole microtubules and is involved in the lengthening of the spindle in late anaphase (anaphase B). The relocalization of CENP-E to the midzone may reflect a role for CENP-E in crosslinking microtubules during spindle elongation.

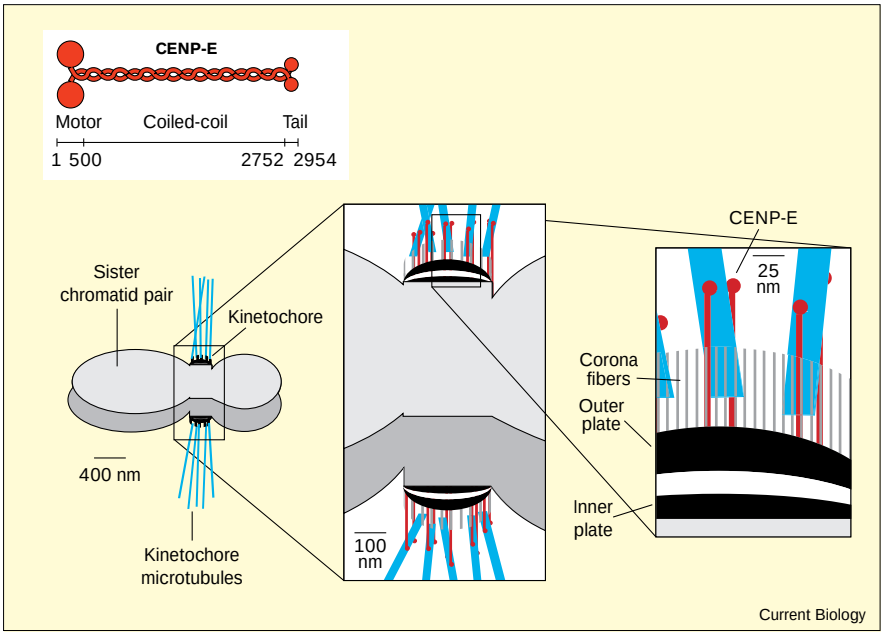
Using immuno-electron microscopy, it was possible to show that CENP-E localizes to the interface between chromatin and microtubules even early in mitosis, when partially-assembled kinetochores have yet to assume mature electron-dense structures. It is reasonable to assume that these sites of CENP-E staining are the sites of nascent kinetochore formation [9,10]. In the subsequent period from prophase through anaphase, kinetochores are visible as distinct trilaminar structures and CENP-E is found in the fibrous region extending from the outer kinetochore plate and overlapping the ends of microtubules [9,10] (Figure 2). Because of its location, this fibrous corona is thought to contain the microtubule binding components of kinetochores.

CENP-E polarity

What is the polarity of the CENP-E motor? A motor's polarity is usually defined as the direction it moves on non-dynamic (usually taxol-stabilized) microtubules in the presence of ATP. Classical kinesin is a plus-end-directed motor, but some kinesin-like motors move towards the minus ends of microtubules; dynein is also a minus-end-directed motor. The oscillatory nature of chromosome movement implies a role for both plus-end-directed and minus-end-directed motor activity at kinetochores. Determining which types of movement are associated with which motors is, however, more tricky than it might appear. For example, in the absence of ATP, plus-end-directed kinesin can actually move cargo towards the minus ends of depolymerizing microtubules, apparently because the motors can hold on as depolymerization occurs (see below).

An early study [11] detected minus-end-directed microtubule motor activity in anti-CENP-E immunoprecipitates. More recently, however, Wood *et al.* [12] have shown that a bacterially-expressed fragment of CENP-E containing the motor domain has plus-end-directed motor activity *in vitro*. One possible explanation for this discrepancy is that the minus-end-directed motor activity brought

Figure 2



The structure (inset) and kinetochore localization (at three magnifications) of the CENP-E motor protein. The CENP-E stalk is potentially long enough for a single dimer to bind to any of the 20–30 microtubules that attach to a typical mammalian kinetochore. See text for details.

down by anti-CENP-E antibodies might have arisen from another motor protein associated with CENP-E. A second possibility is that CENP-E has both plus-end-directed and minus-end-directed motor activity, depending on its state of covalent modification or on its association with regulatory factors.

CENP-E function in the mitotic spindle

To investigate the functions of CENP-E in mitotic chromosome movement, Wood *et al.* [12] immunodepleted the motor from *Xenopus* oocyte extracts, and then examined spindle formation *in vitro*. In oocyte extracts from which CENP-E had been depleted, most sister chromatids failed to align at the metaphase plate. One possible explanation for this is that chromosomes skipped prometaphase and metaphase and entered anaphase prematurely. But sister chromatid separation — a marker for anaphase entry — had not occurred on the misaligned chromosomes, suggesting that CENP-E plays a role in either the movement of chromosomes towards the metaphase plate or maintaining them in that position once they are there.

Taking a different approach, Schaar *et al.* [13] used antibody microinjection to study CENP-E function in living cells. When injected into interphase cells, anti-CENP-E antibodies prevented metaphase chromosome alignment. In injected cells, some chromosomes formed only monopolar attachments and remained near one pole, and other chromosomes formed bipolar attachments but failed to congress. These results generally agree with those in *Xenopus* oocytes and show that, in living cells, CENP-E is necessary to form bipolar spindle attachments and to move bivalently-attached chromosomes to the spindle equator.

Not all chromosome movement was abolished by anti-CENP-E antibodies, however. In injected cells, those chromosomes that had achieved bivalent attachment oscillated between the spindle poles, much like monovalently- and bivalently-attached metaphase chromosomes in unperturbed cells [2]. The existence of this oscillation in CENP-E-depleted cells shows that some aspects of chromosome movement were intact, even though the coordinated movements leading to congression were defective.

A model for CENP-E function

We can account for these observations by postulating that, during congression, the plus-end-directed motor activity of CENP-E is responsible for moving chromosomes along polymerizing microtubules towards their plus ends. It has been demonstrated, however, that pulling forces associated with the tips of shrinking microtubules (minus-end-directed movement), rather than pushing forces at the growing microtubule ends (plus-end-directed movement), provide the main force driving congression [14]. The failure of chromosomes to congress properly when CENP-E activity is compromised suggests that CENP-E plays a role in the minus-end-directed movement of chromosomes, even though it has a demonstrated plus-end-directed motor activity.

How could the plus-end-directed motor activity of CENP-E drive minus-end-directed movement? One possible

mechanism has been demonstrated in experiments by Lombillo *et al.* [15]. *In vitro*, chromosomes isolated from cultured cells bind via kinetochores to microtubule plus ends. When these microtubules are induced to depolymerize and motor movement is prevented by depleting ATP, the chromosomes remain attached to, and follow, the shrinking microtubule ends to generate minus-end-directed movement. This association with a shrinking microtubule is inhibited by adding antibodies against CENP-E. Thus it is possible that, in cells, CENP-E is involved in generating minus-end-directed movement by passively attaching chromosomes to shrinking microtubule ends. An alternative possibility, suggested by the observation that cells microinjected with anti-CENP-E antibodies still support oscillatory movement of chromosomes, is that CENP-E regulates movement towards the equator and is not required for the actual attachment of kinetochores to microtubule ends.

Conclusions

Kinetochore-mediated chromosome movement is dependent on a large number of different proteins, most of which have not yet been identified, that must act in concert to attach chromosomes to microtubules, control the oscillatory movements of prometaphase, maintain chromosome position at the metaphase plate, and move chromosomes poleward at anaphase. The task of studying this movement is confounded by the difficulty of separating the different types of motion *in vitro*, while maintaining a semblance of the motion that chromosomes undergo in living cells. Nevertheless, recent progress with CENP-E demonstrates that the identification and characterization of kinetochore-associated microtubule motor proteins is a good first step in the search for a mechanistic understanding chromosome segregation.

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